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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR INDUCING DIFFERENTIATION OF EMBRYONIC STEM CELLS AND USES THEREOF

(57) Abstract: The present invention provides a method for inducing differentiation of an embryonic stem cell into a differentiated neural cell. The present invention further provides a method for producing differentiated neural cells, and a population of cells comprising the differentiated neural cells. Additionally, the present invention provides a method for repopulating a spinal cord in a subject, and a method for treating nervous tissue degeneration in a subject in need of treatment. The present invention further provides neural progenitor cells, differentiated neural cells, and uses of same. Also provided is a transgenic non-human animal containing the differentiated neural cells. The present invention is further directed to a method for isolating a population of differentiated neural cells. Finally, the present invention provides a method for identifying an agent for use in treating a condition associated with neuron degeneration.

Applicants: Thomas M. Jessell, et al.  
Serial Number: 10/789,308  
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Exhibit 55

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/20399

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/85, 15/86; A61K 38/00, 39/395; C12Q 1/70; G01N 33/53

US CL : 514/1,2; 424/130.1; 435/4, 7.1, 7.21, 325

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/1,2; 424/130.1; 435/4, 7.1, 7.21, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,851,832 A (WEISS et al.) 22 December 1998 (22.12.1998), Table II.	1,26,103
X	US 6,294,346 B1 (WEISS et al.) 25 September 2001 (25.09.2001), Table II, Examples 8-9.	1,26,103
X	US 5,980,885 A (WEISS et al.) 09 November 1999 (09.11.1999), Example 37.	1,26,103
A	US 6,277,820 B1 (ROSENTHAL et al.) 21 August 2001 (21.08.2001), entire document.	1-26, 29-54, 103-105
A	US 5,817,773 A (WILSON et al.) 06 October 1998 (06.10.1998), entire document.	1-26, 29-54, 103-105
A	US 6,040,180 A (JOHE) 21 March 2000 (21.03.2000), entire document.	1-26, 29-54, 103-105
A	GROSS, R.E. et al. Bone Morphogenetic Proteins Promote Astroglial Lineage Commitment by Mammalian Subventricular Zone Progenitor Cells. Neuron. October 1996, Vol. 17, No. 4, entire document.	1-26, 29-54, 103-105

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

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# INTERNATIONAL SEARCH REPORT

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## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LILLIEN, L. et al. BMP and FGF Regulate the Development of EGF-Responsive Neural Progenitor Cells. Development. November 2000, Vol. 127, No. 22, pages 4993-5005, entire document.	1-26, 29-54, 103-105

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/20399

### Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-26,29-54 and 103-105
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☒

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

PCT/US03/20399

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

According to PCT Rule 13.2, unity of invention exists only when the shared same or corresponding technical feature is a contribution over the prior art. The inventions listed as Groups 1-6 do not relate to a single general inventive concept because they lack the same or corresponding special technical feature.

Group 1, claim(s) 1-26 and 29-54, drawn to a method of inducing differentiation of an embryonic stem cell comprising contacting said cell with amounts of a rostralizing and/or caudalizing embryonic signaling factor and a dorsalizing or ventralizing embryonic signaling factor and transplanting said cells.

Group 2, claim(s) 27-28, 55-56, and 95-100 drawn to differentiated neural cells.

Group 3, claim(s) 57-94, drawn to a method for repopulating a spinal cord in a subject.

Group 4, claim(s) 101, drawn to a transgenic non-human animal line.

Group 5, claim(s) 102, drawn to a method for isolating a population of differentiated neural cells.

Group 6, claim(s) 103-105, drawn to a method for identifying an agent.

The inventions listed as Groups 1-6 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature of Group 1 is a method of inducing differentiation of an embryonic stem cell comprising contacting said cell with amounts of a rostralizing and/or caudalizing embryonic signaling factor such as FGF and a dorsalizing or ventralizing embryonic signaling factor such as BMP which is shown by US 5,851,832 (Weiss et al.) 22 December 1998 to lack novelty or inventive step and does not make it a contribution over the prior art. US 5,851,832 teaches the incubation of neurospheres, a small cluster of embryonic stem cells with bFGF, a type of FGF, and BMP-2, a type of BMP (Table II).

Group 1 is drawn to the special technical feature of inducing differentiation of an embryonic stem cell, which is not shared by any of the other groups.

Group 2 is drawn to the special technical feature of differentiated neural cells, which is not shared by any of the other groups.

Group 3 is drawn to the special technical feature of a method for repopulating a spinal cord in a subject, which is not shared by any of the other groups.

Group 4 is drawn to the special technical feature of a transgenic non-human animal line, which is not shared by any of the other groups.

Group 5 is drawn to the special technical feature of a method for isolating a population of differentiated neural cells, which is not shared by any of the other groups.

Group 6 is drawn to the special technical feature of a method for identifying an agent, which is not shared by any of the other groups.

# INTERNATIONAL SEARCH REPORT

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## Continuation of B. FIELDS SEARCHED Item 3:

WEST (USPT, PGPUBS, US OCR, JPO, EPO, DERWENT); NCBI (PUBMED); STN (BIOSCIENCE)  
embryonic stem cell, fetal, embryonic, pluripotent cell, multipotent cell, BMP, FGF, Wnt, RA, SHh